A novel homozygous variation in the *PANK2* gene in two Persian siblings with atypical pantothenate kinase associated neurodegeneration

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Abstract

Pantothenate Kinase-associated Neurodegeneration (PKAN) is an autosomal recessive disorder that is caused by variation in pantothenate kinase-2 gene (*PANK2*) gene on chromosome 20. The common presentation of this disease includes progressive dystonia, Parkinsonism, retinopathy, cognitive impairment, and spasticity. The typical magnetic resonance imaging finding is *eye of the tiger* sign in globus pallidus and not pathogenic and not found in all patients. In the present study, we describe two siblings who have a novel variation of the *PANK2* gene. These patients with the same genotype, have different ages at the onset of disease and also the various severity of the disease. The description of these cases helps to understand this disease, its symptoms, pathogenesis, and its treatment.

Introduction

Neurodegeneration with brain iron accumulation (NBIA) includes a heterogeneous group of hereditary neurodegenerative diseases that occur with symptoms such as dystonia, parkinsonism, cognitive impairment and vision loss.[1-3] The most common type is Pantothenate Kinase-associated Neurodegeneration (PKAN), due to a variation in the pantothenate kinase-2 gene (*PANK2*) gene on chromosome 20.[4] The disease has two types, classic and atypical, the classical type occurs at an earlier age and more severe. The typical MRI finding is eye of the tiger sign that is T2-hypointensity surrounding a central hyperintensity in globus pallidus and not pathogenic and not found in all patients.[5-7] In this paper, we present two siblings of patients with PKAN who have a novel variation of the *PANK2* gene.

Case Report

A 32-year-old woman (case 1) was referred to our clinic because of gait difficulties that had started at the age of about 16 years. The family history, drug history, and past medical history were noncontributory. On neurologic examination, the Mini-Mental State Examination (MMSE) was normal. Cranial nerve examination was normal and the Ophthalmologic evaluation indicated no evidence of visual impairment, Kayser-Fleischer rings, pigmentary retinopathy and atrophy of optic nerve. She had hypokinesia and bradykinesia and limb rigidity, which was more on the right side, also there were generalized dystonia and spasticity of lower limbs without cerebellar signs. Acanthocytosis was not seen in the peripheral blood smear and the other laboratory tests were normal. A brain magnetic resonance image (MRI) revealed the eye of the tiger sign (Figure 1A), and the genetic analysis showed a homozygous variation in the PANK2 gene NM_153638.3:c.706_708delGAA (GAA deletion, p.E236 Del).

The second patient (case 2) was a 30-year-old man presents with generalized dystonia and Parkinsonism that had started at the age of about 18 years. Gradually, the dystonia increased in severity, His developmental history was normal, the ophthalmological examination was normal with no evidence of visual impairment and retinopathy. He also had a generalized dystonia, hypokinesia, and limb rigidity. There were no pyramidal or cerebellar signs. Brain MRI discloses a typical eye-of-the-tiger sign (Figure 1B), and a genetic study detected a pathogenic variation in the *PANK2* gene (GAA deletion, p.E236 Del). For DNA extraction from type of blood cells, Q1AAMP DNA MICROKIT (Cat number: 56304) was used. For comprehensive investigation of all the exons and splicing sites of the introns in *PANK2* gene, primers from another paper were used (Table 1).[8] Briefly, PCR reaction was performed in a final volume of 25 µL containing 100-200 ng of total DNA, 10 pmol of each primer, 2.5 mM MgCl2, 200 mM each of dNTP and 1 U Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany). The reaction mixture was cycled 35 times at 95◦C for 1 min, annealing temperature (°C) for 1 min (refer to Table 1) and 72◦C for 1 min. Electrophoresis of the PCR products was performed on 2% agarose gels (Figure 2). The PCR products were sequenced with the forward or reversed primers on an ABI 3730XL sequencer (Macrogen Company, Korea) and compared with control samples using the FinchTV program and analyzed on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).With these methods, the target sequence for each patient was compared with the normal reference sequence, and mutations in the exons and the splicing sites of the introns in the *PANK2* gene. All transcribed exons of the *PANK2* gene were investigated in patients. delGAA homozygous mutation in the exon2 was detected in both siblings and their parent were heterozygous in this location. Both patients were treated with Madopar 125 three times daily and Deferiprone 500 twice daily, which we did not see any changes in symptoms after a three month follow up.

We obtained the written informed consent from the two patients for publication of the case report

Discussion and Conclusions

Pantothenate Kinase-Associated Neurodegeneration (PKAN) is an autosomal recessive disorder caused by a

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variation in the *PANK2* gene. The clinical manifestations include speech disorder Parkinsonism, dystonia, spasticity, Visual loss, dysphagia seizure, and dementia.[2,3,9] All of the patients with Pantothenate Kinase Associated Neurodegeneration do not have eye-of-the-tiger sign especially in the early stages of this disease and all of the patients with this sign do not have a *PANK2* mutation.[10-13] This gene has seven exons, most variations are missense but duplication, deletion and splice also reported,[6,14,15] in our patients, there is a novel variation that was GAA deletion resulting in the p.E236del that bioinformatically or in silico showed it is probably pathogenic mutation but needs further investigation. Two of our patients had manifestations but the progression, and severity of the disease was different. The course of the disease in the first patient was faster and the symptoms were more severe.

Case 2 had a later onset of symptoms, about 18 years, with slow progression and the severity of the symptoms was milder. Usually Bulbar symptoms, generalized dystonia, and gait disturbance, are more common in classical form and tremors, segmental dystonia, seizure, psychiatric disorders and Parkinsonism more common in atypical form of PKAN disease.[4,16,17] Because the precise mechanism of these diseases has not been discovered so far, most of the treatments used so far reduce the severity of symptoms to some extent.[1,18] The dystonia is debilitating in these patients and is exacerbated over time and is treated with drugs such as Oral and Intrathecal Baclofen, Botulinum Toxin, gabapentin and anticholinergics. Surgery such as Deep brain stimulation, ablative pallidotomy or thalamotomy is also used to treat this disease.[18-21] Iron chelator drugs such as deferiprone have been used in some studies in neurodegenerative diseases and have promising results. This drug passes through the blood-brain barrier and reduces brain iron and reduces the oxidative stress response in the brain.[21-24] In conclusion, although the disease is rare. But familiarity with clinical and radiological symptoms can help to diagnose this disease more easily.

Table 1. Primers for studying the *PANK2* gene by PCR-sequencing.

Primers	Forward/Reverse	Product size (bp)	TM(°C)
1	F: GGAACTAGGCCGAGGGACAAAG R:GAAGGTACCGCTTTCGGAG	1200	63
2	F: GCCCCAAAACCCTTTTGC R: ACCACCTCTAGATGGCCAACTC	650	60
3	F: TGGGTCTGTAGTAGCAGG R: CATTTGTTTGCATAATCCAG	580	54
4	F: GACATGGGCCCTGTGTTTTG R: GGCCCGCCTGTATTTCTTAG	438	60
5	F: GCTCTGTTTGAAGTTTGC R:ATGACTACATTTAGGCACTG	402	54
6	F: TCCTGTGACATTATCTAGCATG R: AGCCCATTTCACCTCCAC	429	54
7	F:TCTGAAGTGCTTGGATAGTC R:CTTCCTGGTTGCTAATTTAG	454	54

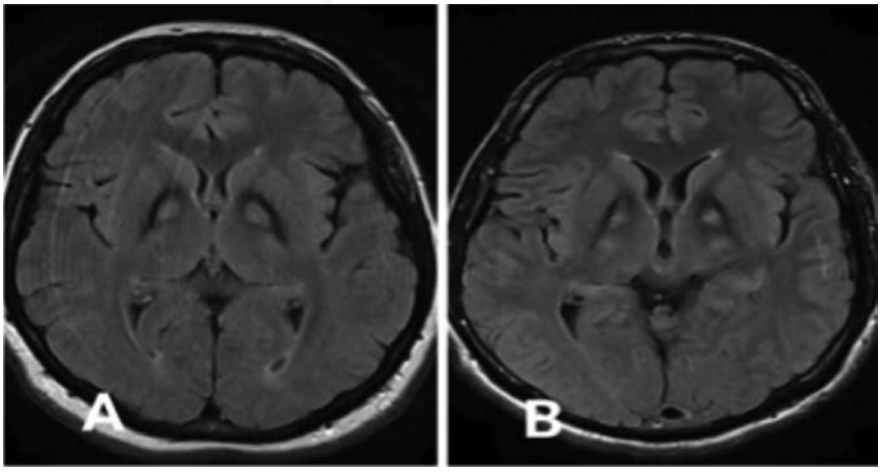

Figure 1. Brain magnetic resonance imaging T2-FLAIR images of case 1 (A) and 2 (B) revealed the eye of the tiger sign.

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